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(54) Title: IL-2R-ASSOCIATED POLYPEPTIDE AND DNA MOLECULES CODING THEREFOR

(57) Abstract

The present invention relates to a polypeptide, p43, which is associated with the interleukin-2 receptor (IL-2R). It binds specifically to the β and γ subunits of IL-2R and is further capable of binding NAD⁺. The invention is further related to nucleic acid molecules coding for p43 and to antibodies specifically binding p43.

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IL-2R-associated polypeptide and DNA molecules coding therefore

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Field of the invention

The present invention relates to the polypeptide p43, to polypeptides which contain binding sites for at least two of NAD^+ , interleukin 2 receptor (IL-2R) β -chain, or IL-2R γ -chain, to nucleic acid molecules containing the coding information for the aforementioned polypeptides, to antibodies specific for the aforementioned polypeptides, to antisense oligonucleotides, to pharmaceutical compositions containing the aforementioned polypeptides or nucleic acids, and to methods of producing the aforementioned polypeptides.

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Background of the invention

Interleukin 2 (IL-2) plays a critical role in the regulation of proliferation and differentiation of hematopoietic cells (27, 29). IL-2 exerts its multiple biological activities through its binding to a specific cell surface receptor (IL-2R) (30), including protein tyrosine kinase (PTK) activation, and nuclear proto-oncogene expression which may be critical for cellular proliferation (16, 29). IL-2R contains at least three distinct subunits; the α -chain, the β -chain and the γ -chain (5, 9, 28). Among these subunits, both the IL-2R β - and γ -chains belong to a newly identified superfamily of cytokine receptors, characterized by four conserved cysteines and the sequence Trp-Ser-X-Trp-Ser (the "WS motif") in their extracellular domains (1, 2). Notably, none of the IL-2R subunits possesses any known catalytic activity such as PTK activity.

The expression of different combinations of the IL-2R subunits gives rise to various forms of IL-2R, each of which exhibiting different binding affinity to IL-2 (28). The "high-affinity" IL-2R (K_d ; 10^{-11}M) consists of the heterotrimer α -, β - and γ -chains, the "intermediate-affinity" IL-2R (K_d ; 10^{-9}) results from the heterodimer β - and γ -chains, whereas the "low-affinity" IL-2R (K_d ; 10^{-8}) can be generated by expression of the α -chain alone. IL-2R β -chain possesses the largest cytoplasmic domain, consisting of 288 amino acids (a.a.) and was shown to play a critical role in IL-2 signal transduction (8). When the human IL-2R β -chain cDNA was introduced into murine IL-3-dependent pro-B cell line BAF-B03, which normally expresses the endogenous IL-2R α - and γ -chains, but not the β -chain, these cells were capable of proliferating in response to IL-2 (3, 8). Further expres-

sion studies with deletion mutant cDNAs of the IL-2R β -chain revealed that a restricted cytoplasmic region of the IL-2R β -chain, designated the "serine-rich" region (S-region), is indispensable for *c-myc* gene induction and for mitogenesis following IL-2 stimulation of the BAF-B03 cells (26). Another cytoplasmic region of the IL-2R β -chain, rich in acidic amino acids, designated the "acidic" region (A-region), is required in addition to the S-region for the *src*-family PTK activation and p21^{ras} activation and for *c-fos/c-jun* gene induction following IL-2 stimulation of BAF-B03 cells (6, 7, 17, 24, 26). Several lines of evidence suggest that the IL-2R γ -chain may also be critical for IL-2-induced signal transduction (29). Moreover, IL-2R γ -chain is suggested to be a shared common component among the IL-2, IL-4 and IL-7 receptors and possibly other cytokine receptors (14, 15, 21, 23). Mutations of IL-2R γ -chain have been found in X-linked severe combined immunodeficiency patients who show defects in T-cell development (22), providing evidence for the critical role of IL-2R γ -chain in cytokine signaling. Furthermore, recent studies have indicated that the functional cooperation between the cytoplasmic domains of IL-2R β -chain and γ -chain is critical for IL-2 signaling (11, 19, 20).

Because of the importance of IL-2R-mediated processes for normal body functions and disease, there is a need of better understanding of these processes as well as the need of new tools for influencing them.

Disclosure of the invention

The present invention provides a new IL-2R-associated protein, p43, and nucleic acid molecules containing the coding information for p43. Preferably, the p43 polypeptide has the amino acid sequence of SEQ ID NO: 2 (cf. Fig. 1A):

Met Glu Phe Leu Lys Thr Cys Val Leu Arg Arg Asn Ala Cys Thr
Ala Val Cys Phe Trp Arg Ser Lys Val Val Gln Lys Pro Ser Val
Arg Arg Ile Ser Thr Thr Ser Pro Arg Ser Thr Val Met Pro Ala
Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
Gln Asn Met Met Met Pro Ile Ile His Tyr Pro Asn Glu Val Ile
Val Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
Leu His Val Lys Ile Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
Tyr Phe Lys Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu

Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
 Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
 Gly Gly Leu Asn Asp Lys Asn Cys Thr Gly Lys Arg Val Leu Ile
 Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
 5 Lys Ala Trp Asp Ala His Val Thr Ala Val Cys Ser Gln Asp Ala
 Ser Glu Leu Val Arg Lys Leu Gly Ala Asp Asp Val Ile Asp Tyr
 Lys Ser Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Pro Phe
 Asp Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala
 Pro Asp Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
 10 Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Ile Ala Asp
 Gly Met Leu Gln Thr Gly Val Thr Val Gly Ser Lys Ala Leu Lys
 His Phe Trp Lys Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
 Ser Gly Pro Cys Leu Asp Asp Ile Ala Glu Leu Val Asp Ala Gly
 Lys Ile Arg Pro Val Ile Glu Gln Thr Phe Pro Phe Ser Lys Val
 15 Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys
 Thr Val Ile Asn Val Val,

or SEQ ID NO: 4 (cf. Fig. 1B, mouse p43):

20 Met Gly Val Leu Lys Thr Cys Val Leu Arg Arg Ser Ala Cys Ala
 Ala Ala Cys Phe Trp Arg Arg Thr Val Ile Pro Lys Pro Pro Phe
 Arg Gly Ile Ser Thr Thr Ser Ala Arg Ser Thr Val Met Pro Ala
 Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
 Gln Asn Met Met Leu Pro Ile Ile His Tyr Pro Asn Glu Val Ile
 25 Ile Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
 Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
 Leu His Met Lys Thr Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
 Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
 Tyr Phe Gln Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
 30 Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu
 Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
 Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
 Gly Gly Leu Ser Asp Arg Asn Cys Lys Gly Lys Arg Ala Leu Ile
 Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
 35 Lys Ala Trp Gly Ala His Val Thr Ala Val Cys Ser Lys Asp Ala
 Ser Glu Leu Val Arg Lys Leu Gly Ala Asp Glu Val Ile Asp Tyr
 Thr Leu Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Leu Cys
 Ala Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala

Leu Asn Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
 Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Val Ala Asp
 Gly Met Leu Gln Thr Gly Val Thr Val Gly Thr Lys Ala Met Lys
 His Leu Trp Gln Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
 5 Ser Gly Pro Tyr Leu Asp Glu Ile Ala Glu Leu Val Asp Ala Gly
 Lys Ile Arg Pro Val Ile Glu Arg Thr Phe Pro Phe Ser Glu Val
 Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys
 Thr Val Val Asn Val Val.

10 The invention is further related to polypeptides with p43-like activity or functional derivatives of p43, especially polypeptides which contain a binding site for at least NAD⁺ and IL-2R β -chain, NAD⁺ and IL-2R γ -chain, or IL-2R β -chain and IL-2R γ -chain. Functional derivatives may be variants, fragments, chemical derivatives, or fusion proteins of p43.

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In a further aspect, the present invention is related to nucleic acid molecules containing the coding information for p43, polypeptides with p43-like activity, or functional derivatives. Preferably, a nucleic acid molecule according to the present invention is a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 1 (cf. Fig.

20

1A):

AGAATGGACA GAATACTGAC TGGAACGTTA ATTCGAGCAT TTCATATGCG
 AAGAGCGGAA TAACAGTTCC GTATTCTTCT TTCAGTTTCT CCATTAGATT
 AGCTTCATTT TCGAAGGCTC CGTTTTGCAT GCTTAATTTT GAAACTAGCC
 25 CGTGGTTTGG CAGAATTGGA CTGAATTCAG GGGTGAGAGT TTGATCCAGT
 CCAAGTGTAT TTGAATTGGA GCACGCAGTT CAACCAAGTGT TTACA
 ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
 GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
 AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
 30 TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
 CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
 GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
 AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
 TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
 35 CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
 TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
 AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
 GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT

TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC
TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC
5 AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT
CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
10 GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
15 ACT GTA ATT AAT GTT GTT TAAATAAAAA TGCAGTTTAG TGATTAAAAA
AAAAAAAAAA AAAAAAAAAA,

or a degenerate variant of said nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 1, or a nucleic acid molecule capable of hybridizing to a nucleic acid molecule having SEQ ID NO: 1, or a nucleic acid molecule containing a part of the
20 nucleotide sequence of any of the foregoing nucleic acid molecules, or a fragment of any one of the foregoing nucleic acid molecules. Preferably, such a nucleic acid molecule containing a part of SEQ ID NO: 1 contains the nucleotide sequence of SEQ ID NO: 9:

25 ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
30 GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
35 AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT
TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC

TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
 AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC
 AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
 AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
 5 GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT
 CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
 GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
 GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
 CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
 10 AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
 AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
 CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
 ACT GTA ATT AAT GTT GTT,

15 or a degenerate variant of SEQ ID NO: 9.

Preferably, a nucleic acid molecule according to the present invention is capable of hybridizing to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 1 under conditions which select for a homology, or sequence identity, of more than 50 %, more preferably more than 70 %, more preferably more than 80 %, more preferably more than 90 %. Preferably, such nucleic acid molecules capable of hybridizing contain the coding information for polypeptides with p43-like biological and/or immunological activity, said polypeptides, more preferably, having at least one of the binding sites of p43 for NAD⁺, IL-2R β -chain, or IL-2R γ -chain, more preferably at least two of said
 25 binding sites.

A further aspect of the present invention is a vector containing the nucleotide sequence of any one of the foregoing nucleic acids, especially when said nucleotide sequence is operationally linked to an expression control sequence as in expression vectors.

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A further aspect of the present invention is a host cell carrying a vector as described, especially an expression vector. Such a host cell can be a procaryotic or eucaryotic cell. Preferably, such a host cell is a bacterial cell, a yeast cell, or a mammalian cell. More preferably, said host cell is an *E. coli* cell or a COS cell.

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Accordingly, a still further aspect of the present invention is a method of production of p43, functional derivatives of p43, or polypeptides with p43-like activity, by recombinant expression. Such a method is characterized by cultivating a host cell as de-

scribed, said host cell carrying an expression vector containing the coding information for p43, a functional derivative of p43, or a polypeptide with p43-like biological activity, under conditions where said coding information is expressed by said host cell, and isolating the expressed polypeptide.

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A further aspect of the present invention is an antibody molecule specific for p43, a functional derivative of p43, or a polypeptide with p43-like activity. Such an antibody molecule can be a polyclonal or monoclonal antibody, a complete immunoglobulin or a fragment thereof, especially a Fab' or F(ab)₂ fragment, a recombinant antibody or antibody fragment, for example a recombinant single-chain antibody (scFv), a chimeric, bi-specific or humanised antibody.

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Preferably, such an antibody molecule is specific for one of the following amino acid sequences:

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SEQ ID NO:10: CKVVQKPSVRRISTTSPRST
SEQ ID NO:11: CYKSGSVEEQLKSLKPFDFI
SEQ ID NO:12: CGGSTETWAPDFLKKWSGAT,

20 SEQ ID NO: 11 being preferred.

A still further aspect of the present invention is an antisense oligonucleotide corresponding to a part of the nucleotide sequence of any nucleic acid molecule according to the present invention. One preferred embodiment of such an oligonucleotide has the sequence SEQ ID NO: 8: 5'-GTCTTCAAAACGCCCATCCT-3'.

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A still further aspect of the present invention is a pharmaceutical composition containing p43, a functional derivative of p43, or a polypeptide with p43-like activity, or a nucleic acid containing the coding information for any one of the foregoing polypeptides, or an oligonucleotide corresponding to a part of the nucleotide sequence of said nucleic acid molecule. Such a pharmaceutical composition can be used for the treatment and diagnosis of IL-2-related disorders.

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As used herein, a "polypeptide with p43-like activity" is a polypeptide which exhibits a biological activity which is essentially similar to p43. This means that it has one or more, preferably at least two of its structural or catalytic properties in common with p43, for example with respect to the binding properties of p43 to NAD⁺, IL-2R β -chain, and/or IL-2R γ -chain. As used herein, a "functional derivative" of p43 is a compound which possesses

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a biological activity (either functional or structural) that is substantially similar to a biological activity of p43. Examples of biological activities include the ability to bind to a natural ligand of p43, preferably to bind at least two of NAD⁺, IL-2R β -chain, or IL-2R γ -chain. A molecule is said to be "substantially similar" to another molecule if both molecules have substantially similar structures or if both molecules possess a similar biological activity. The "functional derivatives" of p43 include fragments, variants, chemical derivatives or fusion proteins of p43. The term "fragment of p43" is meant to refer to any polypeptide subset of that molecule. The term "variant of p43" is meant to refer to a molecule substantially similar in structure to either the entire molecule, or to a fragment thereof, provided that the variant has at least one biological activity that is either similar to an activity of p43 or inhibitory to an activity of p43. A variant of p43 may differ from p43 by the substitution, deletion or addition of one or more amino acids, preferably 1 to 10 amino acids. Preferably, a variant has the ability to bind at least two of NAD⁺, IL-2R β -chain, or IL-2R γ -chain. A "chemical derivative of p43" is a molecule which has been derived from p43 by a chemical reaction, for example iodination, acetylation, or linkage to a radioisotope or toxin. A "fusion protein of p43" is a polypeptide which has been generated by recombinant expression of all or a part of the p43 gene fused to all or part of another gene or nucleic acid containing in-frame coding information. A "degenerate variant" of a nucleic acid molecule is a second nucleic acid molecule which has a different nucleotide sequence as compared to the first nucleic acid molecule and codes for the same amino acid sequence as the first nucleic acid molecule, due to the degeneracy of the genetic code. A "fragment" of a nucleic acid molecule means a second nucleic acid molecule which has a nucleotide sequence representing a part of the nucleic acid sequence of the first nucleic acid molecule.

One way of carrying out the present invention is to isolate cDNAs whose protein products can interact with IL-2R γ -chain. To screen for human cDNA encoding proteins able to interact with IL-2R γ -chain, the two hybrid screening procedure described by (37) and (4) can be employed. In principle, DNA coding for IL-2R γ -chain (28), or a part of it, is fused to a DNA coding for the N-terminal domain of the *Saccharomyces cerevisiae* GAL4 protein, said N-terminal domain being capable of binding to specific DNA sequences (UAS_G). This construct can then be incorporated into an expression vector and transformed into a yeast strain which is deficient in GAL4. A cDNA collection which is to be screened can be incorporated into an expression vector, wherein the individual cDNA molecules are fused to DNA coding for the transcriptional activation domain of GAL4. The resulting constructs are transformed into the same yeast strain which has been pretransformed with the IL-2R γ -chain construct. In yeast cells which carry the cDNAs of interest, namely cDNAs coding for polypeptides able to bind to IL-2R γ -chain, those molecules bind to the IL-2R γ -chain polypeptides which are expressed in the same cell, thus bringing the two GAL4 do-

mains (the DNA binding domain fused to IL-2R γ -chain and the transcriptional activation domain fused to the cDNA of interest) together. As a result of this interaction, transcription of genes regulated by GAL4/UAS_G occurs. This can be employed for a suitable selection system, for example using the well-known β -galactosidase/galactose system. For example, LexA protein and IL-2R γ -chain fused gene can be constructed and transformed into appropriate yeast cells. The resultant transformant cell can be sequentially transformed with a pACT human cDNA library (4), and transformants can be subjected to the screening procedure. Transformants can be placed under selection, and surviving colonies can be screened for their ability to produce β -galactosidase. Positive clones consisting of a partial open reading frame fused to the GAL4 transcriptional activation domain can be identified. Using the cDNA insert of such a positive clone as probe, the overlapping cDNAs can be obtained. A full-length cDNA clone may be obtained or constructed from overlapping fragments by standard procedures. cDNAs obtained this way can then be used to screen other cDNA libraries, for example from other species like mouse, to identify related polypeptides. This can be performed by standard procedures as well.

Given the information of the present invention, especially the sequence information according to Fig. 1A and Fig. 1B, the polypeptides and nucleic acid molecules of the present invention can be produced by standard procedures. A nucleic acid molecule with the nucleotide sequence according to Fig. 1A, for example, can be produced by chemical synthesis. An alternative way would be to chemically synthesize an oligonucleotide or DNA fragment corresponding to a part of the nucleotide sequence as outlined in Fig. 1A and to screen an appropriate cDNA library or genomic library by hybridization. Detailed protocols how to design such an oligonucleotide or DNA fragment, how to generate a library, and how to screen such a library by hybridization with the oligonucleotide or DNA fragment can be found in standard laboratory manuals, for example in (32), especially in chapters 7, 8, 9, 11 and 12, the content of which shall be incorporated into this specification by reference. Therein, it is also taught how to adjust the appropriate hybridization conditions for a given probe, for example conditions which select for perfect matching (homology of 100%), or conditions which select for homologies of 50%, 70%, 80% or 90% (32, pages 11.45-11.57). As an example, using a human p43 cDNA as a probe, hybridization in 3 x SSC at 65°C could select mouse p43 cDNA which has a homology of about 90% on the amino acid level. Alternatively, a nucleic acid containing the coding information for p43, or a fragment thereof, can be generated from a cDNA library by polymerase chain reaction according to standard laboratory protocols (32, chapter 14).

With a nucleic acid coding for p43 or a functional derivative thereof in hands, especially the coding sequence according to Fig. 1A (starting with A at position 246 and ending

with T at position 1433 of the nucleotide sequence of Fig. 1A), the expert can produce the polypeptide by recombinant expression according to standard protocols either in procaryotic or eucaryotic host cells (see, for example, 32, especially chapters 16 and 17). For this purpose, the nucleic acid molecule containing the coding sequence of interest is incorporated into an expression vector where it is operationally linked to an expression control sequence. This expression vector is adapted to the special requirements of the host cell of choice. Expression may be regulatable. The expression vector is then introduced into the host cell of choice. Upon cultivation under appropriate conditions, the host cells synthesize the p43 polypeptide or functional derivative thereof. The expression system may permit secretion of the expressed polypeptide into the culture medium. The polypeptide can then be isolated from either the host cells or, when the expressed polypeptide is secreted into the medium, from the culture medium. Specific examples for the expression of p43 or functional derivatives thereof are described below.

Given the information of the present invention, especially the sequence information of Fig. 1A or Fig. 1B, the expert may construct functional derivatives of p43. This can be achieved by constructing a DNA molecule containing the coding information for a functional derivative, incorporating this DNA molecule into an expression vector, introducing this expression vector into a host cell and then expressing said DNA molecule coding for said functional derivative. For example, the expert can produce a fragment of a DNA molecule coding for p43, said DNA fragment containing only a part of the complete sequence, and express this fragment. For a functional analysis of the resulting polypeptide fragment, the expert can perform binding studies with the natural ligands of p43, NAD⁺, IL-2R β -chain, or IL-2R γ -chain, either as described below, or with similar methods. Preferably, fragments of p43 retain at least one, more preferably at least two of the binding sites for NAD⁺, IL-2R β -chain, or IL-2R γ -chain. For the production of variants, the expert can modify a DNA molecule containing all or part of the complete coding information for p43 by standard procedures, for example site-directed mutagenesis (32, especially chapter 15; 33, chapter 11, p 279-295), and express the thus modified DNA molecule as described. As an example, variants may be characterized by substitution, insertion or deletion of one, two, three, or more amino acids, as compared to p43 as described. After expression, the thus generated variant polypeptide can be tested whether it is functional as described. For the production of chemical derivatives of a given polypeptide, standard procedures may be used as well (see, for example, 33, chapter 9, p 225-245, and chapter 10, p 247-277). The generation of fusion proteins is described in the examples below.

Given the information of the present invention, especially the sequence information according to Fig. 1A or Fig. 1B, the expert can produce antibodies specific for p43, or

functional derivatives thereof, according to standard procedures (34, especially vol. 1, chapters 2, 3, 4). For use as an antigen, for example, a synthetic peptide representing a part of the amino acid sequences of SEQ ID NO: 2 or 4, or Fig. 1A or 1B, can be synthesized and used in an immunization protocol, optionally linked to a carrier. Another example for generating an antigen is the recombinant expression of p43 or a functional derivative thereof, optionally as a fusion protein, for example in *E. coli*. The expressed polypeptide or fusion protein - optionally purified - can then be used in an immunization scheme. Specific antibodies or - in the case of monoclonal antibodies - hybridomas which produce specific antibodies can then be selected by appropriate methods (35). Antibodies may either be monoclonal or polyclonal. Instead of an intact immunoglobulin, fragments of immunoglobulins may be used, for example Fab' or F(ab)₂ fragments. The production of recombinant antibodies or antibody fragments, chimeric antibodies, humanised antibodies, bispecific antibodies or single-chain antibodies for a given antigen are state of the art. Antibodies may be coupled to other agents, for example radioisotopes or toxins. Antibodies specific for p43, or functional derivatives thereof, are useful tools for studying the mechanism of IL-2-induced cellular events or can be used to block or impair the transmission of the IL-2-induced signal.

Antisense oligonucleotides can be chemically synthesized according to standard procedures.

P43, functional derivatives thereof, nucleic acids containing the coding information for p43 or functional derivatives thereof, antibodies specific for p43 or functional derivatives thereof, or antisense oligonucleotides corresponding to parts of the nucleotide sequence coding for p43 or functional derivatives thereof can be used as drugs for the manufacture of pharmaceutical compositions for therapy or diagnosis of IL-2-related disorders. The molecules of the present invention can be formulated according to known methods, wherein these materials are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles, and their formulation, optionally with other human proteins included, e.g. human serum albumin, are described, for example, in (36). The pharmaceutical compositions may be administered to patients intravenously, intramuscularly, subcutaneously, enterally, or parenterally. Administration may be by continuous infusion, or by single or multiple boluses. The dosage will vary depending upon such factors as the patients age, weight, height, sex, general medical condition, disease, etc. In general, it will be in the range of from about 1 pg/kg body weight to 10 mg/kg body weight of patient.

Recombinantly produced p43 or functional derivatives thereof may be also used to study the mechanism of IL-2-induced signal transduction.

Figure legends

Fig. 1. Nucleotide sequence and complete predicted amino acid sequence of p43.

(A) The nucleotide and predicted amino acid sequence of human p43. The amino acid sequence is indicated in single-letter code. Conserved residues of predicted NAD⁺ binding domain are underlined. Nucleotide numbers are on the left, and amino acid numbers on the right. (B) Alignment of human and mouse p43 amino acid sequences. The region of predicted NAD⁺ binding domain shown is boxed.

Fig. 2. Homology between p43 and Dehydrogenase members. Sequences of Alcohol dehydrogenase, Lactate dehydrogenase and Glyceraldehydephosphate dehydrogenase

are aligned to human or mouse p43; numbering is with respect to human p43. Identity with human or mouse p43 is indicated in open boxes.

Fig. 3. p43 mRNA expression in human tissues. Human tissue blot (Clontech, Palo Alto, California). Molecular sizes are indicated on the left (in kilobases).

Fig. 4. Association of p43 with IL-2R in COS cells. (A) Association of p43 with

IL-2R γ -chain. Cell lysates were prepared from COS cells; COS cells transfected with CD4 γ plus LCK tag, CD4 γ plus LCK-p43, CD4 γ M1 plus LCK tag or CD4 γ M1 plus LCK-p43. Aliquots of the respective cell lysates were immunoprecipitated with anti-CD4 antibody (OKT4) followed by anti Lck immunoblotting. (B) Association of p43 with IL-2R β -chain. Cell lysates were prepared from COS cells; COS cells transfected with LCK-p43 plus CD4 γ , CD4 γ M1, CD4 β , CD4 β A or CD4 β S.

Fig. 5. Effect of p43 antisense oligodeoxynucleotide on the IL-2 induced DNA synthesis. FWT-2 cells were analyzed for their ability to incorporate [³H] thymidine in the presence of antisense oligodeoxynucleotide after IL-2 stimulation. The data are represented as the average of triplicate determinations.

Fig. 6. NAD⁺ binds to p43. [³²P] NAD⁺ was incubated with the filter, which was transferred with ovalbumin, the recombinant p43 and alcohol dehydrogenase proteins, in the binding buffer (see Materials and Methods). Then, the filter was washed and exposed.

Examples

Example 1: Two hybrid screening and cDNA isolation

5 Unless otherwise indicated, the protocol was adapted according to (4). For yeast two-hybrid screening, the open reading frame of human IL-2R γ -chain cytoplasmic region (28) was fused to the LexA DNA binding domain in the vector pBTM116 (4) by the following procedure; synthesized oligonucleotide primers (sense: 5'-ATTTCCCGGGGGAACG-GACGATGCCCCGAA-3', antisense: 5'-CTTCTGTCTGACGGATTGGGGTTCAGGTTT-
10 C-3'), which contained SmaI or SalI site, respectively, were used for PCR amplification of the cDNA encoding IL-2R γ -chain cytoplasmic region. The fragment was cut by SmaI and SalI and ligated with pBTM116. The resultant plasmid was cut by PstI and ligated to remove the C-terminal half region of γ -chain. The resultant plasmid was transformed into the CTYLD yeast strain as bait. This transformant strain was sequentially transformed with
15 a B-cell derived pACT human cDNA library (4) which was kindly obtained from Dr. S. Elledge, and 1×10^6 transformants were analyzed by the standard method as described (4). Rare surviving colonies were screened for their ability to produce β -galactosidase. One positive clone was identified. Sequence analysis of the clone termed clone 36 encodes a partial open reading frame of ~1.4 kb fused to the GAL4 transcriptional activation domain.
20 Using this insert as probe, a human cDNA library generated from Jurkat cell mRNA was screened to obtain a full length cDNA for p43 coding sequence. For the isolation of the human full length cDNA, the λ gt11 cDNA library was prepared with poly (A)+ RNA from TPA-induced Jurkat cells (a human T cell leukemia line), according to standard procedures (32). For screening, probe DNA was prepared by XhoI enzyme treatment (cutting) of p43
25 cDNA obtained in the two hybrid screening. Five overlapping clones were characterized and found to possess inserts from 0.5-2 kb. DNA sequencing was carried out using the dideoxynucleotide chain termination method. The clone representing the longest insert coding sequence was sequenced. The clone contained a 2.0 kb cDNA segment that overlapped about 1.4 kb with clone 36, extended about 0.6 kb further to the 5' end, and contained the
30 AUG initiation codon. This revealed a potential open reading frame of 396 amino acid-polypeptide (Fig. 1A) with a predicted molecular size of 43 kd. We called this gene product, p43. A computer-assisted sequence search with the GenBank database revealed that the sequence of p43 bears no significant homology to any known protein, except a partial similarity in a NAD⁺ binding domain.

35

In addition, to obtain the mouse p43 cDNA, a cDNA library generated from mouse spleen cells mRNA was utilized and screened with human p43 cDNA fragment as probe. For the isolation of mouse p43 cDNA clone, the hybridization was performed using the

EcoRI-cleaved insert from the full length human p43 cDNA clone as a probe and using a mouse cDNA library as described (38), and the filters were washed in 3 x SSC at 65°C (SSC buffer was prepared according to ref. 32, p. B.12). Screening of the cDNA library from mouse spleen with human p43 cDNA yielded a clone of highly related sequence. The
5 predicted human and mouse p43 amino acid sequences are highly related, showing ~90 % identity at the protein level (Fig. 1B). The amino acid sequence of NAD⁺ binding domain is conserved in both human and mouse p43 (Fig. 1B and 2).

Expression of p43 mRNA detected by Northern blot analysis was ubiquitous to all
10 human tissues tested. The p43 mRNA is approximately 2.0 kb in length and was most abundant in skeletal muscle (Fig. 3).

Example 2: Association of p43 with IL-2R γ -chain in mammalian cells

15

As mentioned above, we isolated an IL-2R γ -chain associated molecule, p43, using yeast two-hybrid system. However we did not know whether p43 can associate with IL-2R γ -chain in mammalian cells. To confirm the binding of p43 and IL-2R γ -chain in mammalian cells, we constructed two chimeric proteins linking p43 to the specific antibody recognized
20 N-terminal region of p56^{lck} (LCK-p43) and IL-2R γ -chain to the extracellular domain of CD4 (CD4 γ). The expression plasmids, CD4 γ and LCK-p43, were transiently co-transfected into monkey COS7 cells and then the intermolecular association was analyzed by immunoprecipitation with OKT4 and following western blotting analysis with anti-Lck antiserum (for details, see Example 6; see also ref. (7)). LCK-p43 and LCK tag were expressed in the
25 transfected COS cells as assessed by anti-Lck antiserum immunoblotting of whole cell lysates (data not shown). Fig. 4 shows that IL-2R γ -chain bound to LCK-p43, but not to control LCK tag or the truncated IL-2R γ -chain, which contains only the transmembrane region, indicating a direct association of the two proteins in mammalian cells.

30

Example 3: IL-2R β -chain also associates with p43

Because IL-2R γ -chain and β -chain are associated and share important functions in IL-2 signal transduction, we determined whether IL-2R β -chain also associates with p43.
35 To confirm the association of these molecules, we further constructed the chimera genes fusing IL-2R β -chain, or mutant β -chain, to CD4 (CD4-IL-2R β , CD4- β S, CD4- β A). These plasmids and Lck-p43 were cotransfected into monkey COS7 cells and the intermolecular association measured (cf. Examples 2, 6). Fig. 4B shows that p43 can associate with not

only IL-2R γ -chain but also IL-2R β -chain. Interestingly, the p43 was tightly associated with IL-2R β -chain through the S-region which is the critical region for IL-2-mediated signal transduction.

5
Example 4: Synthesis of oligodeoxynucleotides and measurement of [^3H] thymidine incorporation

S-oligodeoxynucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems). The sequence of the sense and antisense oligodeoxynucleotides are:
10 5'-CAGGATGGGCGTTTGAAGA-3' and 5'-GTCTTCAAAACGCCCATCCT-3', respectively. The FWT-2 cell, which is BAF-B03-derived cell line expressing wild-type human IL-2R β - and γ -chains, was used in this experiment. After continuously growing cells were washed with PBS, the cells were distributed into 96-well plates at an initial concentration of
15 1×10^4 per well. An oligomer (5 μM or 10 μM) was added with or without IL-2 (2 nM). After 20 hrs incubation, the cells were pulse-labeled with 1 μCi of [^3H] thymidine (20 $\mu\text{Ci}/\text{mmol}$) (NEN Research Products) 4 hrs prior to harvest.

The effect of p43 sense and antisense oligomers in [^3H] thymidine incorporation after
20 IL-2 stimulation as a parameter of growth was evaluated as shown in Fig. 5. The experiments have been repeated at least three times. It is evident that the p43 antisense oligodeoxynucleotide partially inhibits the [^3H] thymidine incorporation (~30%). On the other hand, no effect was observed using sense oligodeoxynucleotides. These results suggest that p43 molecule alters the IL-2 signal partially, but does not inhibit the full scale signal. It remains to evaluate the presence of redundant associated molecules, which can compensate
25 for the absence of p43 in the presence of antisense oligonucleotides.

Example 5: NAD $^+$ binding assay

30

As mentioned earlier, p43 has partial homology to NAD $^+$ binding proteins, such as alcohol dehydrogenase. To confirm whether p43 can bind NAD $^+$, we performed NAD $^+$ binding assay. We first tested the binding of NAD $^+$ to recombinant p43 produced in bacteria. The complete open reading frame of p43 was fused to 6xHis tag sequences, and the resulting chimeric protein was purified from overexpressing bacterial strains by affinity chromatography on Ni-column. For the recombinant *E. coli* expressing p43, the p43 chimeric protein fused to the 6xHis affinity tag was constructed using vector, 6HisT-pET11d, which
35 was kindly obtained from Mr. Hashimoto (Rockefeller University). Plasmid was trans-

formed into *E. coli* strain BL21/pLysS, and recombinant p43 was purified using Ni-column (Invitrogen). Purified p43 and control proteins (ovalbumin and alcohol dehydrogenase) were applied to SDS-PAGE (10-20 % gradient gel), and electrophoretically transferred onto PVDF membrane filters. After soaking in the binding buffer [50 mM Tris-HCl pH7.5, 1 mM EDTA, 5 mM MgCl₂, 0.3 % (v/v) Tween 20] at room temperature for 1 hr, the membrane filters were incubated with [³²P] NAD in the binding buffer at 20°C for 18 hrs. The filters were then washed with the binding buffer three times and exposed to X-ray film. As shown in Fig. 6, we detected the NAD⁺ binding ability of p43. On the other hand, its binding ability could be completely abolished in the presence of deleted excess cold NAD.

Example 6: Immunoprecipitation and immunoblotting analysis

For immunoprecipitation and immunoblotting of IL-2R and p43, chimeric genes were constructed as follows: the CD4β, CD4βA, CD4βS, CD4γ and CD4γM1 chimeric receptor expression plasmids, which bear CD4 extracellular and transmembrane domains and the cytoplasmic domains of IL-2R β-chain, IL-2R β-chain lacking the internal "S-region" and the "A-region", IL-2R γ-chain, and the membrane proximal 7 amino acids of the cytoplasmic domain of IL-2R γ-chain, respectively, were constructed as described previously (18). Briefly, the CD4β and CD4γ chimeric receptors are comprised of human CD4 extracellular/transmembrane domains, fused in frame with the cytoplasmic domains of IL-2Rβ and IL-2Rγ chains respectively, using the two pairs of synthesized oligonucleotides. The CD4β and CD4γ cDNAs were inserted into the EcoRI/XbaI cleaved pEF vector (25) (pEF-CD4β and pEF-CD4γ) respectively. To construct the expression vectors (pEF-CD4βA and CD4βS) for the chimeric molecules, CD4βA and CD4βS, the pdKCRA and pdKCRS vectors (8), respectively, were digested with NcoI and BamHI. After digestion, the respective NcoI-BamHI fragments (~0.9Kb) were inserted into the NcoI/BamHI-cleaved pEF-CD4β vector. The LCK-p43 chimeric molecule is comprised of the p56^{lck} N-terminal region (~100 amino acids), mutated in the myristilation site from Gly to Ala, fused in frame with p43 using the PCR fragment of LCK. The LCK-p43 cDNA was inserted into pEF vector (25). The constructs were confirmed by restriction enzyme digestion and DNA sequencing.

The experiments for transient cDNA expression studies in COS cells were performed as described previously (7). The immunoprecipitation using anti-CD4 antibody (OKT4) and immunoblotting analysis using anti p56^{lck} antiserum were also performed as described previously (18).

Example 7: Production of antibodies

Polyclonal antibodies have been raised against the following synthetic peptides corresponding to different sequence motifs of p43:

5
SEQ ID NO: 10: CKVVQKPSVRRISTTSPRST (a.a. 23-41)
SEQ ID NO: 11: CYKSGSVEEQLKSLKPFDFI (a.a. 255-273)
SEQ ID NO: 12: CGGSTETWAPDFLKKWSGAT (a.a. 278-296)

10 The protocol of antigenic conjugate preparation, immunization and antibody titer determination is as follows:

The protocol for the conjugation of sulfhydryl-containing peptides to the carrier protein KLH was utilized. In brief, 1 mg of peptide and keyhole limpet hemocyanine (KLH, cf. 15 34, vol. I, p. 26) in 500 μ l of PBS were mixed with 500 μ l of complete Freund's adjuvant using Luer-lock connected syringes. After testing the proper mixing of the conjugate components, rabbits were injected subcutaneously in the back of the neck. The animals were boosted with 500 μ g of antigen (conjugate mixed with incomplete Freund's adjuvant), at intervals of two weeks for a period of three months.

20 The antiserum was periodically tested using an enzyme-linked immunosorbent assay (ELISA), where the peptide KLH conjugate at 1 μ g/ μ l was coated into ninety-six well microplates in coating buffer (0.1 M NaHCO₃ pH 9.0). After washing the microplate with rinse buffer (PBS, 0.1% Tween 20), different dilutions of the immune sera or control sera 25 were added in a volume of 50-100 μ l to the wells and incubated at room temperature for two hours.

30 The plates were washed with rinse buffer and 50 μ l of goat anti-rabbit IgG conjugated with alkaline phosphatase in PBS and 1% BSA and 0.1% Tween 20 was added to the wells and incubated for two hours at room temperature. The microplate was washed with rinse buffer and 100 μ l of substrate, p-phenyl phosphate disodium in substrate buffer was added to the wells. The microplate was incubated at room temperature for 1-2 hours and the optical density (OD) at 405 nm measured (ref. 620 nm).

35 The three different anti-peptide antibodies could recognize the peptide conjugate at a dilution of 1/10⁴. The antibody against peptide SEQ ID NO: 11 could also recognize the *E. coli* expressed recombinant human p43.

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Claims

1. A polypeptide with p43-like biological activity.
- 5 2. A polypeptide containing a NAD⁺ binding domain and a binding site for the IL-2R β -chain.
3. The polypeptide of claim 2 which contains an additional binding site for IL-2R γ -chain.
- 10 4. A polypeptide containing a NAD⁺ binding domain and a binding site for the IL-2R γ -chain.
5. A polypeptide containing a binding site for IL-2R β -chain and a binding site for
15 IL-2R γ -chain.
6. The polypeptide of claims 3 or 5, wherein said polypeptide binds stronger to IL-2R β -chain than to IL-2R γ -chain.
- 20 7. The polypeptide of any one of claims 1 to 6 wherein said polypeptide has the amino acid sequence of SEQ ID NO: 2:

Met Glu Phe Leu Lys Thr Cys Val Leu Arg Arg Asn Ala Cys Thr
 Ala Val Cys Phe Trp Arg Ser Lys Val Val Gln Lys Pro Ser Val
 25 Arg Arg Ile Ser Thr Thr Ser Pro Arg Ser Thr Val Met Pro Ala
 Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
 Gln Asn Met Met Met Pro Ile Ile His Tyr Pro Asn Glu Val Ile
 Val Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
 Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
 30 Leu His Val Lys Ile Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
 Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
 Tyr Phe Lys Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
 Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu
 Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
 35 Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
 Gly Gly Leu Asn Asp Lys Asn Cys Thr Gly Lys Arg Val Leu Ile
 Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
 Lys Ala Trp Asp Ala His Val Thr Ala Val Cys Ser Gln Asp Ala

S r Glu Leu Val Arg Lys Leu Gly Ala Asp Asp Val Ile Asp Tyr
Lys Ser Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Pro Phe
Asp Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala
Pro Asp Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
5 Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Ile Ala Asp
Gly Met Leu Gln Thr Gly Val Thr Val Gly Ser Lys Ala Leu Lys
His Phe Trp Lys Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
Ser Gly Pro Cys Leu Asp Asp Ile Ala Glu Leu Val Asp Ala Gly
Lys Ile Arg Pro Val Ile Glu Gln Thr Phe Pro Phe Ser Lys Val
10 Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys
Thr Val Ile Asn Val Val,

or SEQ ID NO: 4:

15 Met Gly Val Leu Lys Thr Cys Val Leu Arg Arg Ser Ala Cys Ala
Ala Ala Cys Phe Trp Arg Arg Thr Val Ile Pro Lys Pro Pro Phe
Arg Gly Ile Ser Thr Thr Ser Ala Arg Ser Thr Val Met Pro Ala
Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
Gln Asn Met Met Leu Pro Ile Ile His Tyr Pro Asn Glu Val Ile
20 Ile Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
Leu His Met Lys Thr Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
Tyr Phe Gln Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
25 Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu
Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
Gly Gly Leu Ser Asp Arg Asn Cys Lys Gly Lys Arg Ala Leu Ile
Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
30 Lys Ala Trp Gly Ala His Val Thr Ala Val Cys Ser Lys Asp Ala
Ser Glu Leu Val Arg Lys Leu Gly Ala Asp Glu Val Ile Asp Tyr
Thr Leu Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Leu Cys
Ala Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala
Leu Asn Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
35 Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Val Ala Asp
Gly Met Leu Gln Thr Gly Val Thr Val Gly Thr Lys Ala Met Lys
His Leu Trp Gln Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
Ser Gly Pro Tyr Leu Asp Glu Ile Ala Glu Leu Val Asp Ala Gly

Lys Ile Arg Pro Val Ile Glu Arg Thr Phe Pro Phe Ser Glu Val
 Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys
 Thr Val Val Asn Val Val.

5 8. A polypeptide having the amino acid sequence of SEQ ID NO: 2 or 4 or a functional derivative of said polypeptide, said functional derivative binding at least one of NAD⁺, IL-2R β -chain, or IL-2R γ -chain.

9. The functional derivative of claim 8, wherein said functional derivative is a variant, a fragment, a chemical derivative, or a fusion protein of said polypeptide.
 10

10. A nucleic acid molecule containing the coding information for a polypeptide or functional derivative according to any one of claims 1 to 9.

15 11. A nucleic acid molecule containing the nucleotide sequence according to SEQ ID NO: 1:

AGAATGGACA GAATACTGAC TGGAACGTTA ATTCGAGCAT TTCATATGCG
 AAGAGCGGAA TAACAGTTCC GTATTCTTCT TTCAGTTTCT CCATTAGATT
 20 AGCTTCATTT TCGAAGGCTC CGTTTTGCAT GCTTAATTTT GAAACTAGCC
 CGTGGTTTGG CAGAATTGGA CTGAATTCAG GGGTGAGAGT TTGATCCAGT
 CCAAGTGTAT TTGAATTGGA GCACGCAGTT CAACCAGTGT TTACA
 ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
 GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
 25 AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
 TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
 CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
 GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
 AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
 30 TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
 CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
 TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
 AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
 GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT
 35 TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
 GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC
 TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
 AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC

AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT
CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
5 GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
10 CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
ACT GTA ATT AAT GTT GTT TAAATAAAAA TGCAGTTTAG TGATTAAAAA
AAAAAAAAAA AAAAAAAAAA,

or a degenerate variant of said nucleic acid molecule, or a nucleic acid molecule capable of
15 hybridizing to a nucleic acid molecule having said nucleotide sequence, or a nucleic acid
molecule containing a part of SEQ ID NO:1 or a part of any of the aforementioned nucleic
acid sequences, preferably a nucleic acid molecule containing a nucleotide sequence accor-
ding to SEQ ID NO: 9:

20 ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
25 GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
30 AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT
TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC
TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
35 AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC
AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT

CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
5 AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
ACT GTA ATT AAT GTT GTT.

10 or a degenerate variant of SEQ ID NO: 9.

12. A nucleic acid molecule according to claim 11, said nucleic acid molecule being capable of hybridizing to a nucleic acid molecule having the nucleotide sequence according to SEQ ID NO: 1 under conditions which select for a homology of more than 70%, more preferably more than 90%.

13. The nucleic acid molecule of claim 12, said nucleic acid molecule being capable of hybridizing in 3 x SSC at 65°C to a nucleic acid molecule containing a nucleotide sequence according to SEQ ID NO: 1.

20

14. A polypeptide coded by a nucleic acid molecule according to any one of claims 11 to 13.

15. A vector containing the nucleotide sequence of a nucleic acid molecule according to any one of claims 10 to 13.

25

16. The vector of claim 15, said vector being an expression vector.

17. A host cell carrying a vector according to claims 15 or 16.

30

18. Method of production of a polypeptide according to any one of claims 1 to 9 or 14, comprising the steps of

a) cultivating a host cell according to claim 17 under conditions where said polypeptide is expressed by said host cell, and

35

b) isolating said polypeptide.

19. The method of claim 18, wherein said host cell is an *E. coli* cell or a mammalian cell, preferably a COS cell.

20. An antibody molecule specific for a polypeptide or functional derivative according to any one of claims 1 to 9.

21. The antibody molecule of claim 20 which is polyclonal antibody, a monoclonal antibody, a complete immunoglobuline or a fragment thereof, the Fab' or F(ab)₂ fragment of an immunoglobuline, a recombinant antibody or a recombinant antibody fragment, a recombinant single-chain antibody (scFv), a chimeric, bispecific or humanised antibody.

22. The antibody molecule of claim 20 or 21, said antibody molecule being specific for any one of the following amino acid sequences:

15	SEQ ID NO:10:	CKVVQKPSVRRISTTSPRST,
	SEQ ID NO:11:	CYKSGSVEEQLKSLKPFDFI,
	SEQ ID NO:12:	CGGSTETWAPDFLKKWSGAT.

23. An antisense oligonucleotide corresponding to a part of the sequence of a nucleic acid molecule according to any one of claims 10 to 13.

24. The antisense oligonucleotide of claim 23 which has the nucleic acid sequence SEQ ID NO: 8: 5'-GTCTTCAAAACGCCCATCCT-3'.

25. The use of an antisense nucleotide according to claims 23 or 24 for the inhibition of IL-2 dependent cell growth.

26. A pharmaceutical composition containing an antisense nucleotide according to claims 23 or 24.

30

27. A pharmaceutical composition containing a polypeptide according to any one of claims 1 to 9, or 14.

28. A pharmaceutical composition containing a nucleic acid molecule according to any one of claims 10 to 13, 15 or 16.

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AGAAATGACAGAACTACTGACTGGAACGTTAATTCGAGCATTTTCATATGCCAAGAGCGGAATAACAGTTCCGTATTCTTC
TTTCAGTTTCTCCATTAGATTAGCTTCATTTCGAAGGCTCCGTTTTCATGCTTAATTTTGAAGCTAGCCCGTGGTTT
GCCAGAAATTTGACTGAATTCAGGGGTGAGAGTTTGATCCAGTCCCAAGTGTATTGAAATTTGAGCAGCAGTTTCAACCCAG
TGTTTACA

246 H E F L K T C V L R R H A C T A V 17
ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT OCA TGC ACT GCG GTT

297 C F W R S K V V Q K F S V R R I S T T S 37
TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT AGA AGG ATT AGT ACT ACC TCT

357 F R S T V M P A W V I D K Y G K M E V L 57
CCA AGG AGC ACT GTC ATG CCT GCT TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT

417 R F T Q M M M M P I I H Y P N E V I V K 77
CGA TTC ACT CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT GTC AAA

477 V N A A S V M P I D V M M R S G Y G A T 97
GTT CAC GCT OCC AGT GTA AAT CCT ATA GAC GTT AAT ATG AGA AGT GGT TAT GGA GCT ACA

537 A L N M K R D P L H V K I K G E E F P L 117
GCT TTA AAT ATG AAG CGT GAT CCT TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG

597 T L G R D V S G V V H E C G L D V R Y F 137
ACT CTG GGT GCG GAT GTC TCT GCG GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA TAC TTC

657 K P G D E V W A A V P P M K Q G T L S E 157
GAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CTG TGG AAA CAA GGC ACT CTT TCA GAG

717 F V V V S G N E V S H K P K S L T H T Q 177
TTT GGT GTA GTC AGT GGG AAT GAG GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA

777 A A S L P Y V A L T A W S A I M K V G G 197
GCT GGC TCT TGT CCA TAT GTG GCT CTC ACA GGC TGG TCT GCT ATA AAC AAA GTT GGT GGC

837 L M D E N C T G K R V L I L G A S G G V 217
CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC TTA GGC GCT TCA GGC GGA GTT

897 G T F A I O V M E A N D A M V T A V C S 237
GGT ACT TTT GCT ATG CAG GTA ATG AAA OCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC

957 Q D A S E L V R K L G A D D V I D Y K S 257
CAA GAT GCC AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC AAA TCT

1017 G S V E E Q L K S L K P F D F I L D M V 277
GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT GAT TTT ATC CTT GAT AAT GTT

1077 G G S Y E T W A P D F L K K M S G A T Y 297
GGC GGA TCC ACT GAA ACA TGG GCT CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT

1137 V T L V T P F L L M M D R L G I A D G M 317
GTG ACT TTG GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT GGC ATG

1197 L Q T G V T V G S K A L K N F W E G V N 337
TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG CAT TTC TGG AAA GGA GTC CAT

1257 Y R M A F P M A S G P C L D D I A E L V 357
TAT CCG TGG GCA TTT TTC ATG GCC AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG

1317 D A G K I R P V I E Q T F P F S K V P E 377
GAT GCG GGA AAG ATC CCG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT CCA GAA

1377 A F L K V E R G N A R G K T V I N V V 396
GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG ACT GTA ATT AAT GTT GTT TAA

1437 ATAAAAATOCAGTTTAGTGATTAATAAAAAAAAAAAAAAAAAAAAA

Fig 1A

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Human p43	MEFLKTCVLR R NACTAVCFW RSKVVQKPSV RRISTTSPRS TVMPAWVIDK YGKNEVLRF	60
	1??111111 12112111 1221221122 1211111211 11111111 11111111	
Mouse p43	MGVLKTCVLR RSACAAACFW RRTVIPKPPF RGISTTSARS TVMPAWVIDK YGKNEVLRF	60
Human p43	QNMMPPIIHY PNEVIVKVHA ASVNPIDVNM RSGYGATALN MKRDPLHVKI KGEEFPLTLG	120
	11121111 11112111 11111111 11111111 11111111 11111111	
Mouse p43	QNMMLPIIHY PNEVIKVHA ASVNPIDVNM RSGYGATALN MKRDPLHMKI KGEEFPLTLG	120
Human p43	RDVSGVMEC GLDVKYFKPG DEVWAAVPPW KQGTLSFVV VSGNEVSHKP KSLTHTQAAS	180
	11111111 11111111 11111111 11111111 11111111 11111111	
Mouse p43	RDVSGVMEC GLDVKYFQPG DEVWAAVPPW KQGTLSFVV VSGNEVSHKP KSLTHTQAAS	180
Human p43	LPYVALTAWA AINKVGGLND KNCTGKRVLI LGASGGVGTF AIQVMKAWDA HVTAVCSQDA	240
	11111111 11111111 11111111 11111111 11111111 11111111	
Mouse p43	LPYVALTAWA AINKVGGLSD RNCKGKRALI LGASGGVGTF AIQVMKAWGA HVTAVCSQDA	240
Human p43	SELVRKLGAD DVIDYKSGSV EEQLKSLKPF DFILDNVGGS TETWAPDFLK KWSGATYVTL	300
	11111111 11111111 11111111 11111111 11111111 11111111	
Mouse p43	SELVRKLGAD EVIDYTLGSV EEQLKSLKLC AFILDNVGGS TETWALNFLK KWSGATYVTL	300
Human p43	VTPFLLNMDR LGIADGMLQT GVTVGSKALK HFWKGVHYRW AFFMASGPCL DDIAELVDAG	360
	11111111 11111111 11111111 11111111 11111111 11111111	
Mouse p43	VTPFLLNMDR LGVADGMLQT GVTVGTKAMK HLWQGVHYRW AFFMASGPYL DEIAELVDAG	360
Human p43	KIRPVIEQTF PFSKVPEAFL KVERGHARGK TVINVV	396
	11111111 11111111 11111111 11111111 11111111	
Mouse p43	KIRPVIERTF PFSEVPEAFL KVERGHARGK TVNVV	396

Fig 1B

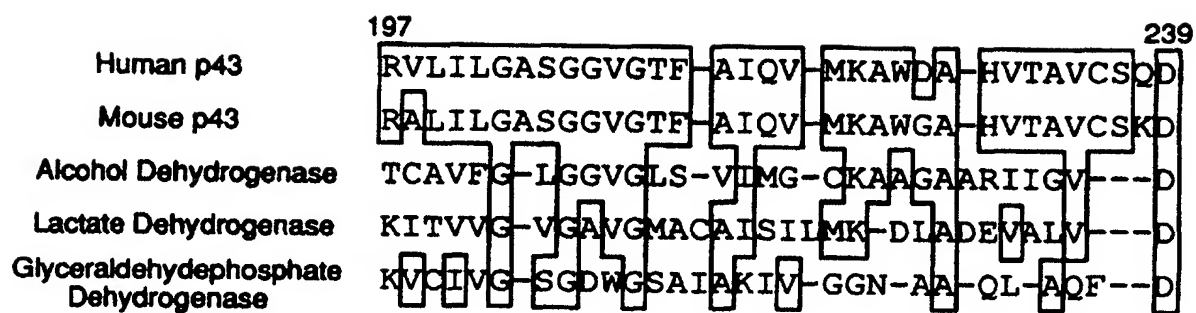


Fig 2

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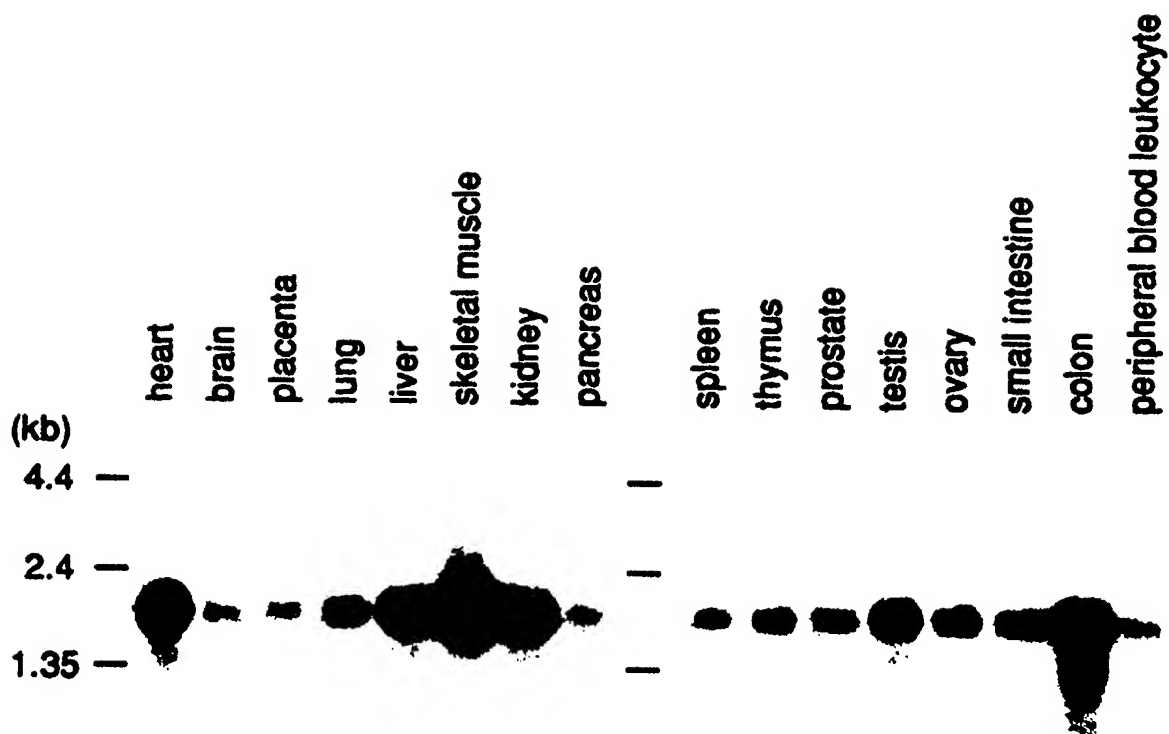


Fig 3

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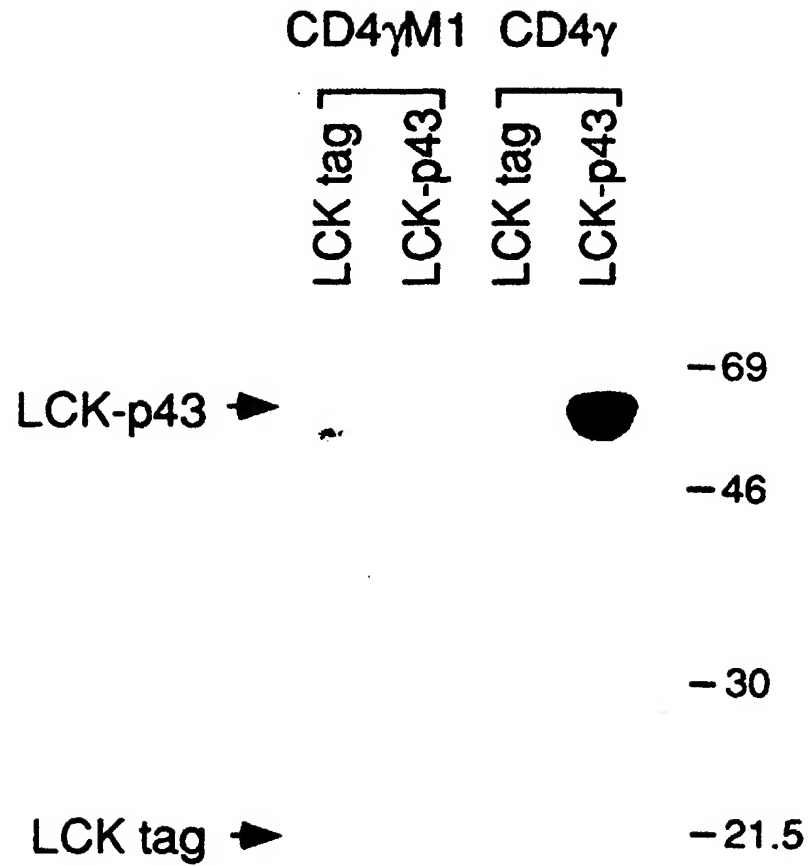


Fig 4A

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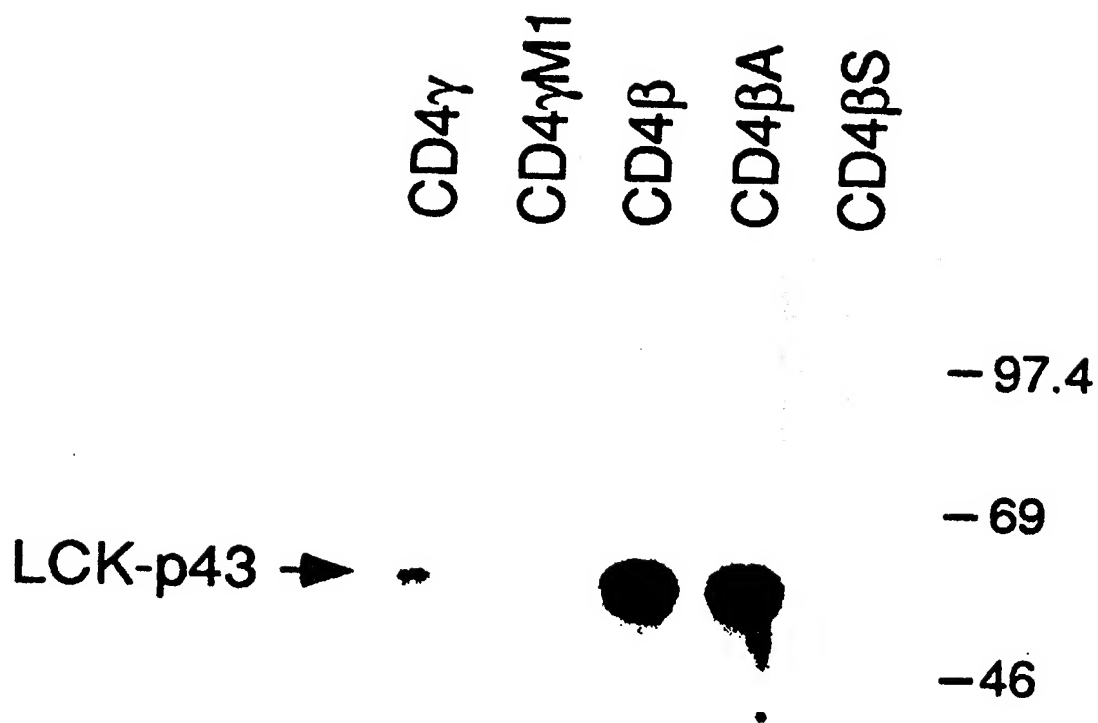


Fig 4B

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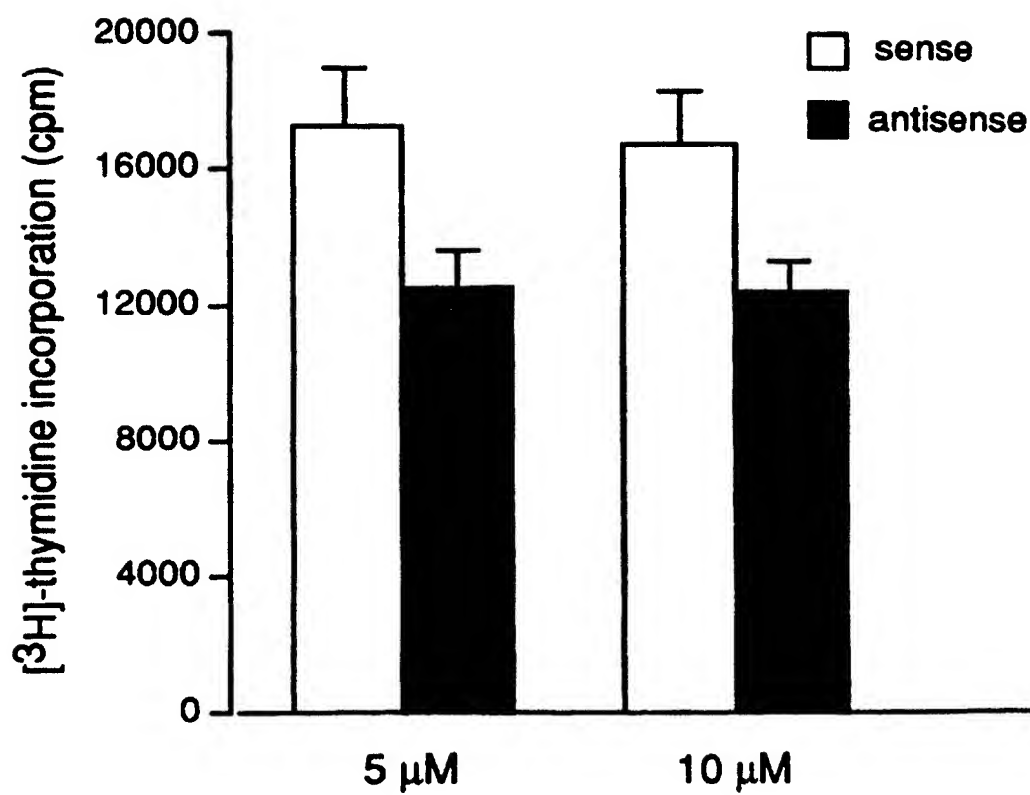


Fig 5

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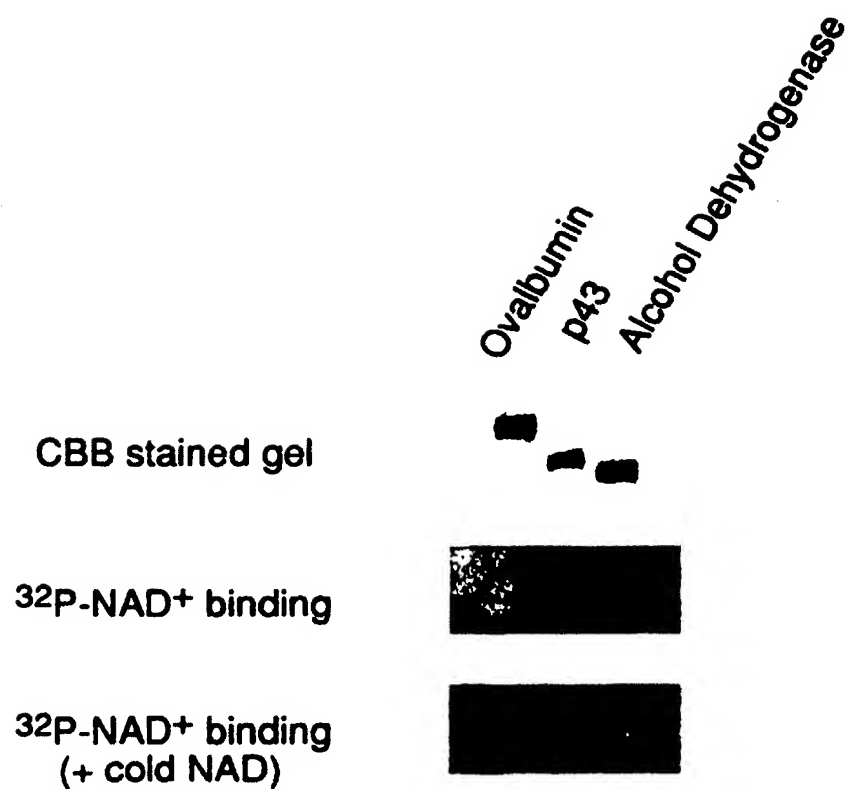


Fig 6

INTERNATIONAL SEARCH REPORT

PCT/EP 95/05123

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 6	C12N15/12 C12N1/20	C07K14/715 C12N5/10	C07K14/47 C07K16/28	C12N15/70 A61K31/70	C12N15/85
According to International Patent Classification (IPC) or to both national classification and IPC					
B. FIELDS SEARCHED					
Minimum documentation searched (classification system followed by classification symbols)					
IPC 6 C07K					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category *	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.
A	EP,A,0 621 338 (AJINOMOTO) 26 October 1994 see claims -----				1
<input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.					
* Special categories of cited documents :					
"A" document defining the general state of the art which is not considered to be of particular relevance			"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention		
"E" earlier document but published on or after the international filing date			"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone		
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)			"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.		
"O" document referring to an oral disclosure, use, exhibition or other means			"A" document member of the same patent family		
"P" document published prior to the international filing date but later than the priority date claimed					
Date of the actual completion of the international search			Date of mailing of the international search report		
16 April 1996			23.04.96		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016			Authorized officer Delanghe, L		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 95/05123

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 25
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: As far as claim 25 is directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the compound.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/EP 95/05123

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-621338	26-10-94	JP-A- 7313188	05-12-95

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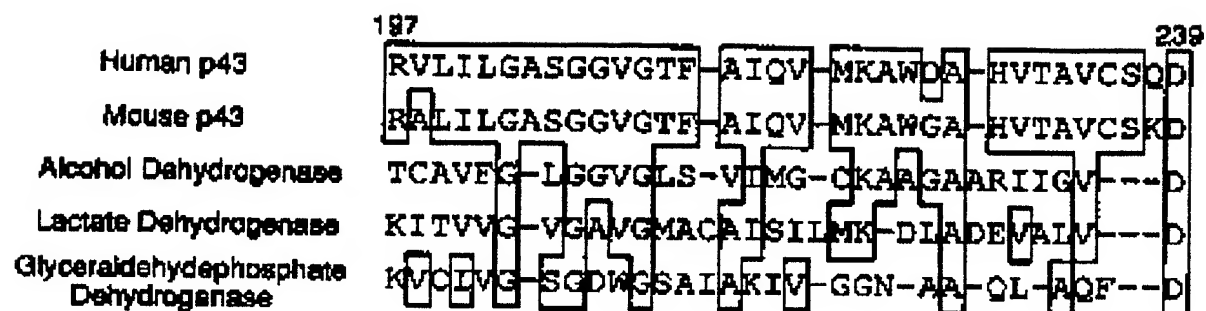
141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200

Fig 1A

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Human p43	MEFLKTCVLR RRACTAVCFW RSKVVDKPSV RRISTTSPRS TVMPAMVIDK YGKNEVLRFET	60
Mouse p43	MGVLKTCVLR RSACAAACEW RRTVIPKPEF RGISTTSARS TVMPAMVIDK YGKNEVLRFET	60
Human p43	QNMCMPIIKY PHEVIKVNHA ASVNPIDVNM RSGYGATALN MKRDPLHVKI KGESEPTLTG	120
Mouse p43	QNMMLPIIHY PHEVIKVNHA ASVNPIDVNM RSGYGATALN MKRDPLHVKI KGESEPTLTG	120
Human p43	RDVSGVVMHC GLDVKYFKPG DEWAAVPPW KQGTLSFVW VSGNEVSHKP KSLTHTQAAS	180
Mouse p43	RDVSGVVMHC GLDVKYFQPG DEWAAVPPW KQGTLSFVW VSGNEVSHKP KSLTHTQAAS	180
Human p43	LPYVALTANS AINKUGGLND KNCYGRVLI LGASGGVGTG AIQVMKAWDA HVTAVCSQDA	240
Mouse p43	LPYVALTANS AINKUGGLSD RNCYGRALI LGASGGVGTG AIQVMKAWDA HVTAVCSQDA	240
Human p43	SELVRKLGAD DVIDYKSGSV EQQLKSLKPF DFILDNVGGG TETWAPDFLK KWSGATYVTL	300
Mouse p43	SELVRKLGAD EVIDYTLGSA EQQLKSLKLC AFILDNVGGG TETWALNFKL KWSGATYVTL	300
Human p43	VTPFLLNMDR LGVADGMLQT GVTVGSKALK HFWKGVHYRN AFFMASGPCL DDIAELVDAG	360
Mouse p43	VTPFLLNMDR LGVADGMLQT GVTVGSKAMK HFWKGVHYRN AFFMASGPCL DDIAELVDAG	360
Human p43	KIRPVIEQTF PFEKVPEAFL KVERGHARGK TVVNVV	396
Mouse p43	KIRPVIERTF PFEKVPEAFL KVERGHARGK TVVNVV	396

Fig 1B



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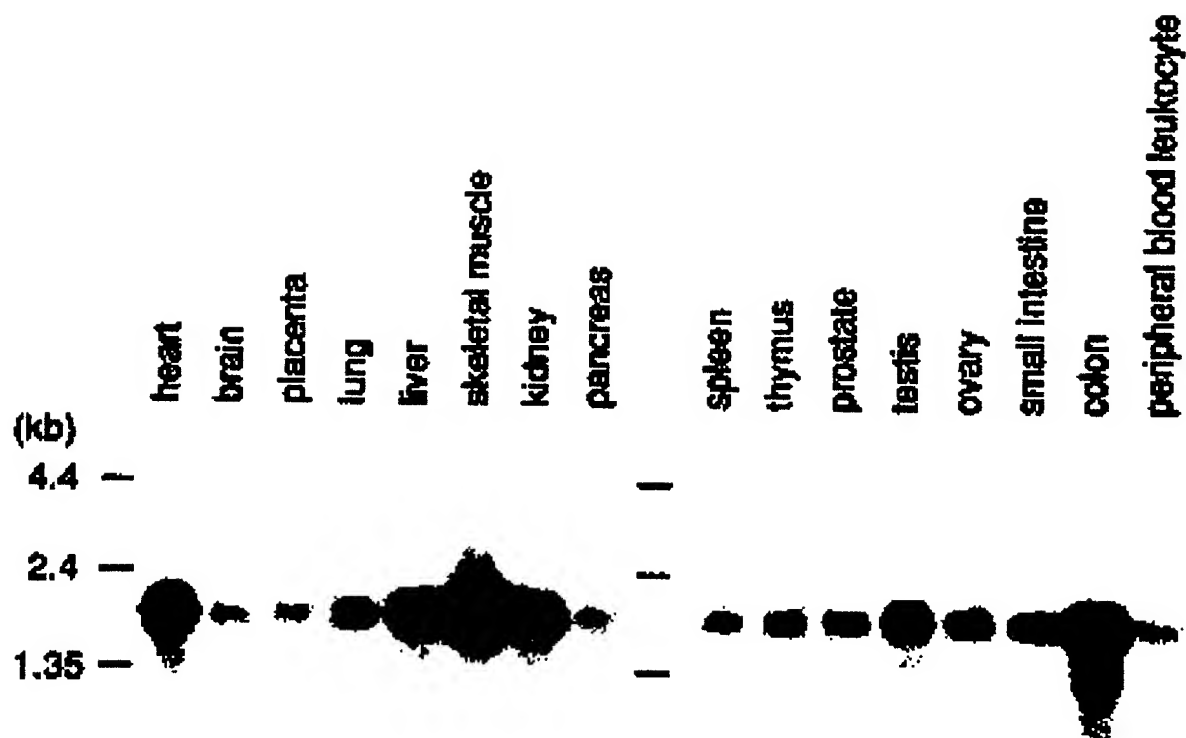


Fig 3

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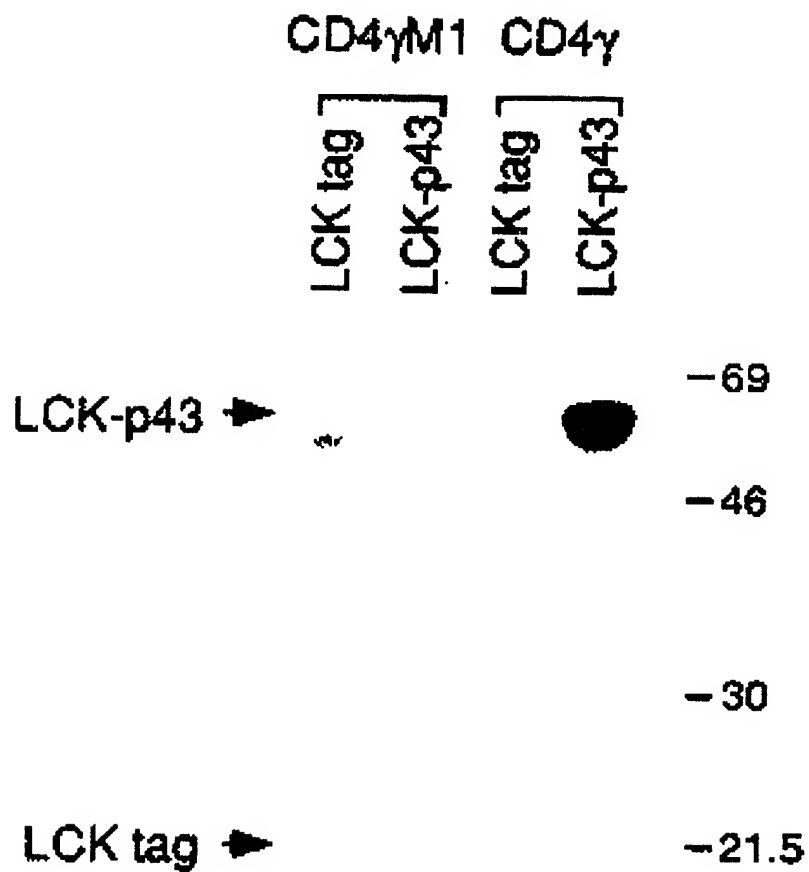


Fig 4A

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Fig 4B

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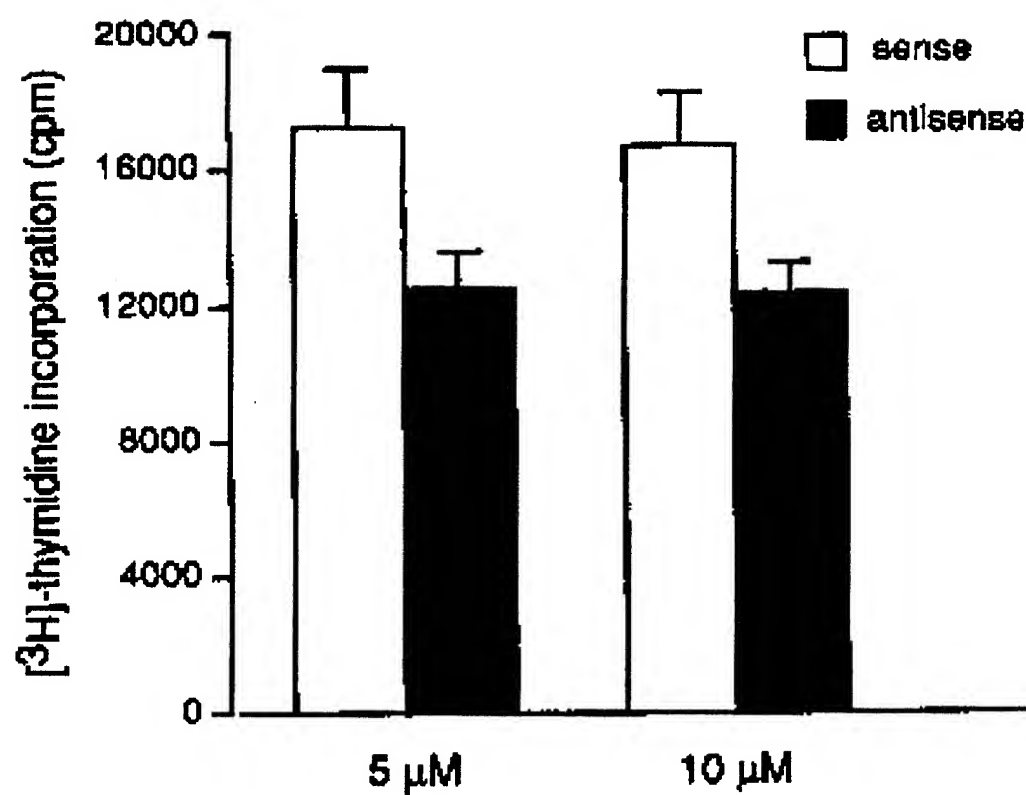


Fig 5

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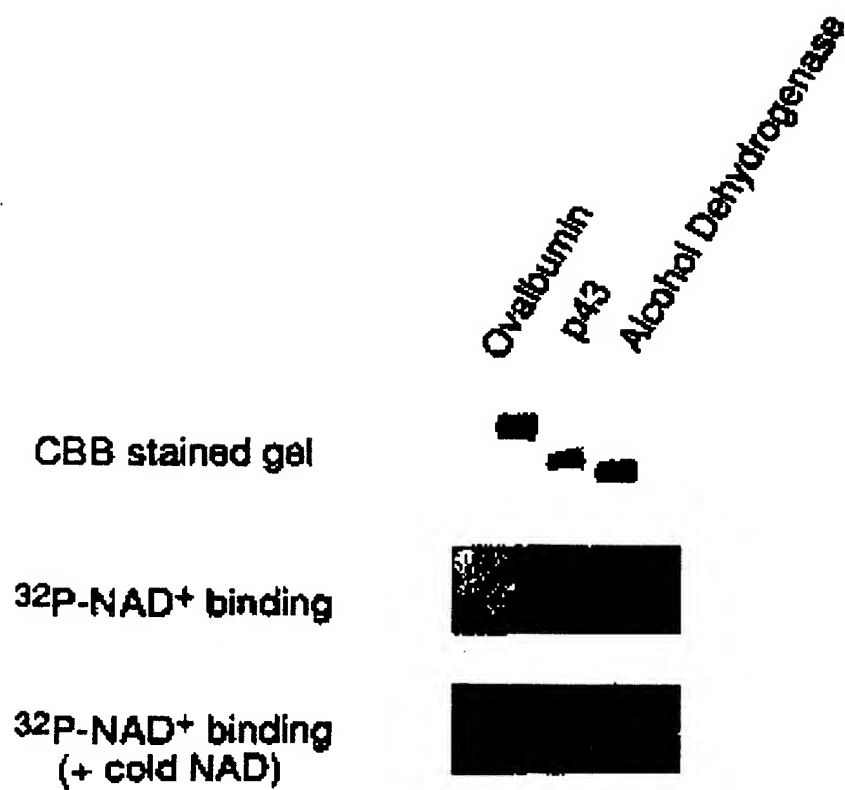


Fig 6